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The impact of a parasitic nematode, *Thripinema fuscum*, on the feeding behavior and vector competence of *Frankliniella fusca*

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Abstract

Frankliniella fusca (Hinds) (Thysanoptera: Thripidae) is the predominant thrips species found inhabiting and reproducing in peanut, *Arachis hypogaea* L. (Fabaceae), and is one of at least seven thrips species reported to transmit Tomato spotted wilt virus (TSWV). The entomogenous nematode *Thripinema fuscum* Tipping & Nguyen (Tylenchida: Allantonematidae), a natural enemy of *F. fusca*, parasitizes larval and adult populations under field conditions. All known *Thripinema* species render the host female thrips sterile and have the potential to suppress pest populations to near extinction. As a result, secondary spread of TSWV in peanut is reduced. Reduction of the virus under field conditions may also be due to lower transmission rates caused by parasite-induced alterations in host feeding behavior. Therefore, the feeding rates of healthy and parasitized *F. fusca* male and female cohorts on leaf discs were recorded daily for 10 days and digital images were subjected to image analysis and viral transmission rates were compared daily using double antibody sandwich enzyme-linked immunosorbent assay. *Thripinema fuscum* reduced the feeding of female *F. fusca* by nearly 65%, and the ability of females to transmit TSWV by 50%. Potential mechanisms underlying the parasite-induced alterations in feeding behavior and transmission are discussed. Parasitism by *T. fuscum* significantly reduced male longevity, but female longevity was not affected. These results provide further evidence that *T. fuscum* aids in regulating viruliferous *F. fusca* pest populations and suggests its potential as a biological control agent for inoculative release in peanut.

Introduction

The tobacco thrips, *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae), is a polyphagous insect pest that feeds on agriculturally important plants in North America (Lewis, 1997). It is one of at least seven thrips species capable of transmitting Tomato spotted wilt virus (TSWV; Bunyaviridae: *Tospovirus*) in crops such as peanut, tobacco, tomato, pepper, as well as in numerous ornamentals, grasses, and weeds (Parrella et al., 2003; Whitfield et al., 2005). *Frankliniella fusca* populations contain both brachypterous and macropterous adults with the proportion of each wing form changing seasonally (Chamberlin et al., 1992). Ecologically, wing form plays a role in thrips dispersal (Chamberlin et al., 1992; Groves et al., 2003), yet, no studies have investigated whether wing form has a direct effect on TSWV replication and transmission.

Transmission of tospoviruses by viruliferous adult thrips is the only significant form of inoculation during natural epidemics and the transmission rate is dictated primarily by the feeding behaviors exhibited by the respective vector (Culbreath et al., 2003; Ananthakrishnan & Annadurai, 2007). TSWV is transmitted in a propagative manner by thrips (Ullman et al., 1993; Wijkamp et al., 1993). Acquisition of TSWV occurs when first and early second instars feed on infective plant tissue, and transmission occurs via late instar and adult thrips that have acquired the virus as larvae (van de Wetering et al., 1996; Wijkamp et al., 1996b). Larvae and adults feed by piercing plant cells and sucking out the cell fluids, which produces

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a silvery scarring on the plant tissue (Hunter & Ullman, 1989). It has been difficult to resolve a consistent pattern in the pathological effects of tospovirus infection on thrips as there have been reports of negative (Stumpf & Kennedy, 2005), neutral (Wijkamp et al., 1996a), and positive (Maris et al., 2004; Stumpf & Kennedy, 2007) effects on thrips. Recent work has shown that genetic variation of both thrips populations and *Tospovirus* isolates, as well as the quality of infected host plant tissue, are important factors in pathogenicity (Stumpf & Kennedy, 2005, 2007). Because vector transmission varies based on both the quality and quantity of feeding of thrips (van de Wetering et al., 1998, 1999), we hypothesize manipulation of feeding behaviors may provide an avenue for decreasing the spread of tospoviruses in agroecosystems.

The insect parasitic nematode *Thripinema fuscum* Tipping & Nguyen (Tylenchida: Allantonematidae) renders female *F. fusca* sterile without any negative effects on their survival (Sims et al., 2005). Parasitism significantly reduces the longevity of male *F. fusca* and the effects of *T. fuscum* on male reproduction are unknown (Sims et al., 2005). Parasitism by *T. fuscum* is initiated when a free-living female enters the host through the intersegmental membranes of the coxal and/or abdominal cavities (Tipping et al., 1998). Once inside the host, the parasitic female produces eggs that hatch and develop through three juvenile stages before boring into the alimentary tract and emerging via the anus as the fourth-stage adult (Sharga, 1932). All stages of *F. fusca* can be parasitized, with young adult females the most preferred (67%) and males least preferred (25%) in laboratory experiments (Sims et al., 2005). Parasitism of adult *F. fusca* has been reported to exceed 80% under field conditions (Funderburk et al., 2002). Parasitism of *F. fusca* larvae on peanut averaged 49 and 28% in laboratory and field experiments, respectively (Sims et al., 2005). The lower rate of larval parasitism under field conditions is most likely due to differences in microhabitat between the larvae and parasitized females; larvae remain within the terminal buds of peanut and parasitized adults typically aggregate in the flowers where they feed on pollen and where free-living *T. fuscum* are able to easily contact new hosts.

Recent observations suggest that parasitism by *Thripinema* spp. may suppress TSWV transmission by reducing host feeding rates (Sims et al., 2005). Related research has shown that the feeding by *Frankliniella occidentalis* (Pergande) is suppressed significantly by the nematode *Thripinema nicklewoodi* Siddiqi; however, the effects of parasitism on vector competence are unclear (Arthurs & Heinz, 2003; Lim & Van Driesche, 2004). Arthurs & Heinz (2003) reported that TSWV infection of *F. occidentalis* did not affect susceptibility to *T. nicklewoodi*, but fewer parasitized thrips became active transmitters and their per capita frequency of disease transmission was reduced by 50%. Alternatively, Lim & Van Driesche (2004) reported that parasitized *F. occidentalis* did not subsequently acquire Impatiens necrotic spot virus as readily as their non-parasitized counterparts, but that rates of transmission remained the same between the two viruliferous groups.

The interaction between *F. fusca*, *T. fuscum*, peanut, and TSWV serves as an ideal multi-trophic system to examine the impact(s) of a chronic disease on vector competence. The first objective of our research was to examine the effects of gender, wing form, virus infection, and nematode parasitism on the feeding behavior of *F. fusca*. The second objective was to determine what effects gender, age, and nematode parasitism have on TSWV transmission. From these experiments, we provide a framework for better understanding how the highly host specific *T. fuscum* parasite interfaces with plant viruses/insect vector associations.

Materials and methods

Maintenance of *Frankliniella fusca*, TSWV inoculum, and *Thripinema fuscum*

Healthy and parasitized *F. fusca* were collected in Alachua Co., FL, USA (29°38′N, 82°21′W), and maintained as described by Sims et al. (2005). Leaves of peanut [*Arachis hypogaea* L. (Fabaceae)] showing TSWV symptoms were collected in Marion Co., FL, (29°24′N, 82°06′W), USA, and confirmed to be TSWV positive by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), a technique used to determine the presence or absence of TSWV by detecting viral structural (nucleocapsid) proteins (SRA 39300; Agdia, Elkhart, IN, USA).

Tomato spotted wilt virus was maintained in peanut by *F. fusca* transmission. For virus acquisition, first instars were placed on infected tissue and allowed to feed for 24 h. Larvae were then transferred to 15-cm polypropylene containers with a 5-cm diameter ventilation hole covered with fine mesh. The polypropylene containers were stored in a sealed plastic crisper lined with moist paper towel and maintained at 27 °C and L14:D10 photoperiod. Fresh tetrafoliate leaves were deposited into containers every day until adult emergence. Transmission of the virus to 'Florunner' peanut was achieved by allowing viruliferous adults to feed for 72 h on 3–6-week-old healthy plants enclosed in cages (12.7-cm-diameter clear plastic cylinders with 6.3- cm-diameter screen holes). Host plants were held at 25–30 °C in a greenhouse. After an incubation period of approximately 10–20 days following the end of the inoculation access period, symptomatic plants were confirmed to be TSWV positive using DAS-ELISA.

Effect of gender, wing form, virus infection, and nematode parasitism on survivorship, feeding behavior, and TSWV transmission

Approximately 200 females were confined for 48 h in four cages containing TSWV-infected peanut for oviposition. Newly eclosed first instars were allowed to feed on TSWV-infected leaves for a 48-h acquisition period to generate viruliferous thrips. After this 48-h period, half of the larvae from each cage were transferred in groups (n = 20) to 1.5-ml microcentrifuge tubes containing two *T. fuscum*-parasitized adult female *F. fusca* excreting nematodes and a 1-cm-diameter peanut leaf disc. Larvae were held with parasitized females for 72 h to achieve optimal levels of parasitism. The remaining half of the larvae were transferred to tubes with two healthy adult females and a 1-cm-diameter peanut leaf disc for 72 h to serve as non-parasitized controls. Corresponding control thrips (i.e., uninfected by TSWV) were reared in the same manner, except for being given healthy rather than TSWV-infected peanut leaves.

Individual larvae were then placed (after the 48-h virus acquisition period and 72-h parasitization access period) in tubes with a fresh peanut leaf disc until adult emergence. After thrips emerged as adults, they were each transferred individually (n = 213) to a new tube that was provisioned with a fresh peanut leaf disc (1 cm^2) with the top (adaxial) surface placed upward every 24 h until death. Immediately after each 24-h feeding period, the upper surfaces of the discs were photographed for feeding injury (Auto-Montage Pro 5.02.0096; Syncroscopy, Frederick, MD, USA) and analyzed (SigmaScan Pro 4.02; Jandel Corporation, San Raphael, CA, USA) using a modified color-defined protocol of Kerguelen & Hoddle (1999) that detected the amount of silvered areas caused by thrips feeding. Background noise was accounted for by subtracting the mean pixel count of unfed control leaf discs from the pixel count of leaf discs with feeding injury. Pixel counts were converted to area of feeding (in mm^2). After measurements were taken, leaf discs were placed on sterile water in 24-well plates for 5 days at 25 °C to amplify the virus titer. Leaf discs were then stored at −70 °C until analysis by DAS-ELISA. Leaf discs were recorded as positive if their ELISA (optical density) value was greater than the determined threshold value for the plate [mean of control leaf disc readings + 3(SD)].

At death, thrips were dissected and the gender, wing form, and presence of *T. fuscum* were recorded. Dissected thrips were then stored individually in tubes with 150 μl of phosphate buffered saline-polyvinylpyrrolidone buffer at −80 °C until further analysis could be conducted using antigen-coated plate (ACP)-Indirect ELISA to confirm the presence of viral replication in adults. The monoclonal antibody probes (Ascites cell lines 1C1A7 lot# A16714 and ascites cell line 6B1C1 lot# A16779; Agdia) used in these assays detect a non-structural protein (NSs) encoded by the small RNA segment of TSWV, thus differentiating thrips that are capable vectors, in which the virus is actively replicating, from those that have merely ingested the virus by feeding on infected plant tissue (Bandla et al., 1994).

Statistical analysis

Survivorship. Thrips were classified according to gender and *T. fuscum* parasitism, giving four treatment groups for non-viruliferous thrips (non-parasitized females, parasitized females, non-parasitized males, parasitized males) (n = 144). However, because only two parasitized, viruliferous males were obtained, which died within 1 day, only three treatment groups were available for viruliferous thrips (non-parasitized and parasitized females, non-parasitized males) (n = 67). This classification enabled us to make similar analyses for viruliferous and non-viruliferous thrips.

Survival distribution curves according to gender, virus infection, and nematode parasitism were generated using Kaplan–Meier estimates (Proc LIFETEST; SAS, 2004), and the Cox proportional hazards model (Proc TPHREG; SAS, 2004) was used to determine how survival rates of adults differed among the four treatment groups of non-viruliferous thrips (non-parasitized and parasitized females, non-parasitized and parasitized males) and among the three treatment groups of viruliferous thrips (non-parasitized and parasitized females, non-parasitized males). Comparisons were made to examine the impact of parasitism status and gender on host longevity.

Feeding behavior. A subset of 10 individuals per treatment (five of each wing form) that survived 10 days or more were randomly selected (RANDBETWEEN function, Excel 2000) for the feeding and transmission analyses. It was not possible to determine the parasitism and viral status of thrips until after all experiments had been conducted, therefore, we were unable to have an equal number of thrips for all categories. The effects of independent variables on the amount of feeding were assessed using a mixed model repeated measures ANOVA with data normalized via a log (y + 1) transformation before analysis (Proc MIXED; SAS, 2004). The daily amount of feeding for each individual thrips was the repeated measure. Because of the potential serial correlations in feeding within each thrips, we used a first-order autoregressive covariance structure in the models. Due to the lack of viruliferous males parasitized by *T. fuscum*, initial analyses were run separately for females and males to determine the effects of wing form, virus infection, and parasitism on feeding injury. These initial models showed that wing form and virus infection did not affect feeding by males and females and were therefore taken out of the analyses. Further analyses (slice option within Proc MIXED; SAS, 2004) were conducted to determine the effects of gender and nematode parasitism, and their interaction on feeding over time.

Transmission rates. Mean cumulative distribution plots were generated for each treatment to observe transmission over time (Proc RELIABILITY; SAS, 2004). To determine whether overall rates of transmission varied (i.e., number of days with transmission/total days of adulthood), comparisons of transmission frequencies among the three groups of viruliferous thrips (non-parasitized and parasitized females, non-parasitized males) were conducted using a generalized linear mixed model ANOVA (Proc GENMOD; SAS, 2006). In this case, transmission was a binary response variable because each observed leaf disc was either infected or not infected. The generalized linear mixed model accounted for the response variable being expressed as the proportion of leaf discs infected relative to the number of leaf discs observed for each individual (Madden et al., 2002). Pairwise comparisons among the treatment groups were made using the LSMeans option.

To determine whether the likelihood of transmission changed over the course of a thrips' lifetime, and whether this was related to their gender or parasitism status, we analyzed the gap times from one transmission event to the subsequent one for the three types of viruliferous thrips (non-parasitized females and females, non-parasitized males) (Johnston & So, 2003; Nelson, 2003; Proc TPHREG; SAS, 2004]. The robust sandwich estimate for the covariance was used to account for potential correlations in gap times within individuals.

To determine whether the amount of feeding each day affected the likelihood of a thrips transmitting TSWV on that day, we analyzed whether the probability of transmission increased with the amount of feeding for each of the three viruliferous thrips states. For these analyses, we used a logistic analysis, with a repeated effect being the 10 daily observations for each thrips (Proc GLIMMIX; SAS, 2006). Amount of feeding was log transformed before analysis. Analyses were conducted separately for the three groups of viruliferous thrips because of differences in the amount of feeding among the groups (see Results below).

Results

Survivorship

Longevity of non-viruliferous thrips was first compared to determine whether *T. fuscum* parasitism affected the longevity of females and males (Figure 1A). Contrasts to compare longevity of parasitized and non-parasitized thrips of each gender showed that females did not differ in their longevity (χ^2 = 0.08, d.f. = 1, P = 0.78), but parasitism led to a significant reduction in longevity among males

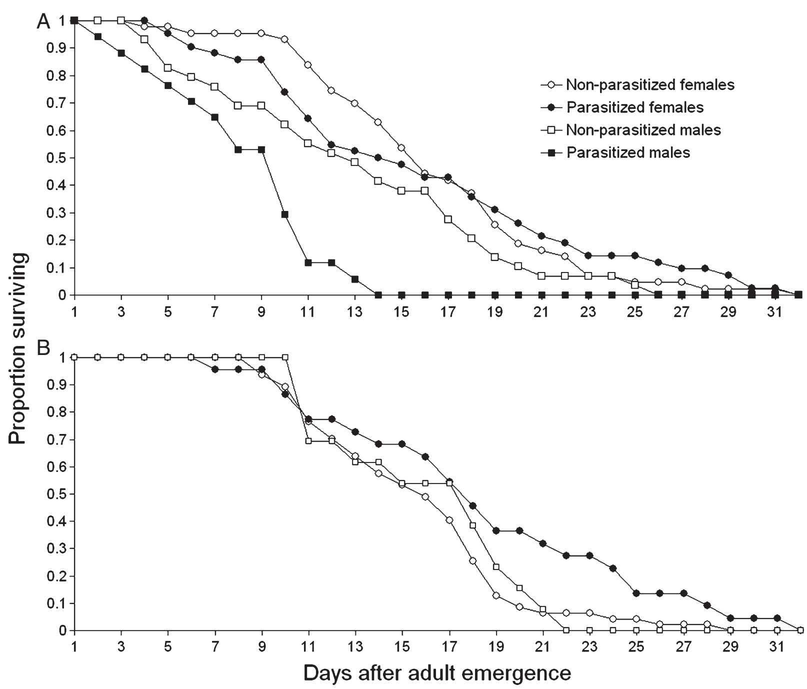

Figure 1 Proportion of (A) non-viruliferous (n = 144) and (B) viruliferous (n = 69) *Frankliniella fusca* individuals surviving throughout adulthood.

Table 1 Mean longevity (± SE) of adult *Frankliniella fusca*

Parasitism status	Viruliferous[1]	Longevity (days)	
		Females	Males
Not parasitized	No	15.15 ± 0.74 (52)	13.17 ± 1.06 (30)
	Yes	15.21 ± 0.72 (39)	15.4 ± 1.4 (10)
Parasitized	No	14.74 ± 1.03 (46)	8.25 ± 0.72 (16)
	Yes	17.72 ± 1.51 (18)	1.0 ± 0 (2)

Numbers in parentheses represent the total numbers of thrips per cohort.
[1]Thrips were categorized as viruliferous if they tested positive for the NSs protein of TSWV by ACP-Indirect ELISA.

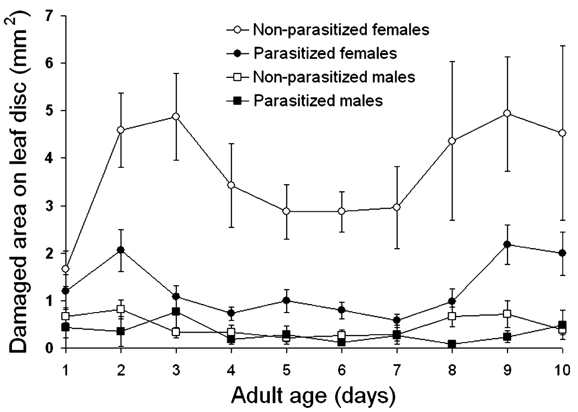

Figure 2 Daily damaged area (mean ± SE) on leaf discs (in mm^2) fed on by *Frankliniella fusca* individuals for the initial 10 days of adulthood (n = 64).

(χ^2 = 16.77, d.f. = 1, P<0.0001) (Table 1). The proportional hazard ratios were similar between each comparison except for parasitized males; there was a 78% chance that a parasitized male would die before a non-parasitized male. The survival distribution graphs showed no difference in longevity between non-viruliferous and viruliferous *F. fusca* cohorts, however, the onset of mortality occurred earlier for viruliferous thrips than for non-viruliferous cohorts (Figure 1). Longevity did not differ between viruliferous non-parasitized (χ^2 = 0.55, d.f. = 1, P = 0.46) and parasitized (χ^2 = 0.67, d.f. = 1, P = 0.41) female thrips (Figure 1B, Table 1).

Feeding behavior

Feeding rates for female *F. fusca* were much higher than for males ($F_{1,60}$ = 119.34, P<0.0001) (Figure 2). There was a significant interaction between thrips gender and parasitism status ($F_{1,60}$ = 16.24, P = 0.0002) on the feeding behavior of *F. fusca* adults indicating that the effect of parasitism on feeding differed between females and males. Parasitized females fed significantly less than non-parasitized females ($F_{1,38}$ = 54.82, P<0.0001); however, there was no significant difference in the feeding rates of parasitized and non-parasitized males ($F_{1,22}$ = 1.25, P = 0.28). There were no significant differences in the mean feeding rates within genders between brachypterous and macropterous individuals, nor within genders between viruliferous and non-viruliferous individuals (Table 2).

Because the three-way interaction between gender, parasitism, and time ($F_{9,540}$ = 0.84, P = 0.58) was not significant and the resulting model with two-way interactions had a lower Akaike's Information Criterion, the three-way interaction was deleted from the analysis. The lack of an interaction suggested that differences in feeding between parasitized and non-parasitized thrips within each gender were consistent over time. There was no significant gender*time interaction ($F_{9,540}$ = 1.33, P = 0.22), indicating

Table 2 Mean total area of feeding (± SE) on leaf discs (mm^2) fed on by *Frankliniella fusca* individuals for the initial 10 days of adulthood

Parasitism status	Viruliferous[1]	Wing form	Total feeding over 10 days (mm^2)	
			Females	Males[2]
Not parasitized	No	Brachypterous	3.60 ± 0.76 (5)	0.72 ± 0.13 (5)
		Macropterous	4.37 ± 0.80 (5)	0.35 ± 0.10 (5)
	Yes	Brachypterous	3.07 ± 0.38 (5)	0.30 ± 0.05 (5)
		Macropterous	3.80 ± 0.68 (5)	0.51 ± 0.13 (4)
Parasitized	No	Brachypterous	1.32 ± 0.21 (5)	0.31 ± 0.08 (3)
		Macropterous	1.15 ± 0.16 (5)	0.34 ± 0.13 (2)
	Yes	Brachypterous	1.33 ± 0.25 (5)	–
		Macropterous	1.24 ± 0.21 (5)	–

Sample sizes are given between parentheses (total n = 64).
[1]Thrips were categorized as viruliferous if they tested positive for the NSs protein of TSWV by ACP-Indirect ELISA.
[2]No parasitized viruliferous males survived beyond 1 day.

that females and males showed similar day-to-day variation in feeding (Figure 2).

Transmission rates

There were significant differences in transmission rates of TSWV among the three groups of viruliferous thrips (χ^2 = 46.49, d.f. = 2, P<0.0001). Non-parasitized females transmitted more than non-parasitized males (χ^2 = 19.54, d.f. = 1, P<0.001). Parasitism significantly reduced virus transmission by females (χ^2 = 41.48, d.f. = 1, P<0.0001). There were no significant differences in transmission rates of parasitized females and non-parasitized males (χ^2 = 1.52, d.f. = 1, P = 0.22). There were significant differences in the gap times between transmissions among the three groups of viruliferous thrips (χ^2 = 13.78, d.f. = 2, P = 0.001), with viruliferous non-parasitized females having a 67 and 74% chance of transmitting before a viruliferous parasitized female and a viruliferous non-parasitized male, respectively. There were also significant differences in the time during thrips life that transmission occurred (χ^2 = 41.34, d.f. = 1, P<0.0001). The mean cumulative distribution plots showed for all treatments that the frequency of transmissions decreased with age; older thrips did not transmit as efficiently as younger thrips (Figure 3, Table 3). The hazard ratio for the overall comparison was ~0.90, so the likelihood of a thrips transmitting TSWV decreased about 10% for each additional day of adulthood. This decline in transmission with age was consistent across categories of thrips (χ^2 = 0.76, d.f. = 2, P = 0.68), reinforcing the idea that parasitism reduced transmission throughout adulthood. Non-parasitized females transmitted at a greater rate than both parasitized females and non-parasitized males, and these differences were consistent throughout the thrips lifetime (Figure 3).

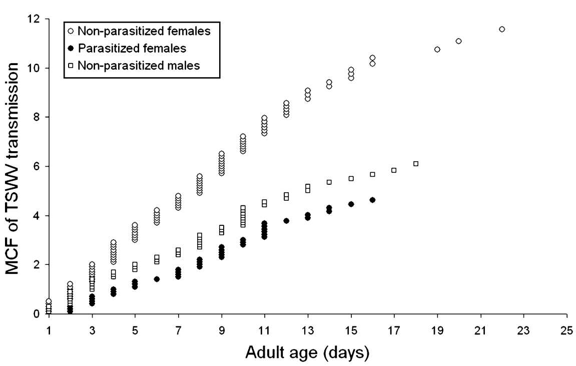

Figure 3 Mean cumulative frequency (MCF) of TSWV transmission to individual leaf discs over the lifetime of adult *Frankliniella fusca.* The flattening of all three curves indicates that the frequency of transmission decreases with thrips age.

Table 3 Proportion of viruliferous *Frankliniella fusca* cohorts transmitting TSWV each day

Day	Non-parasitized females	Parasitized females	Non-parasitized males
1	0.5 (10)	0 (10)	0.22 (9)
2	0.7 (10)	0.3 (10)	0.56 (9)
3	0.8 (10)	0.4 (10)	0.44 (9)
4	0.9 (10)	0.3 (10)	0.33 (9)
5	0.7 (10)	0.3 (10)	0.33 (9)
6	0.6 (10)	0.1 (10)	0.33 (9)
7	0.5 (10)	0.4 (10)	0.11 (9)
8	0.8 (10)	0.4 (10)	0.67 (9)
9	0.8 (10)	0.5 (10)	0.33 (9)
10	0.7 (10)	0.3 (10)	0.78 (9)
11	0.86 (8)	0.67 (9)	0.33 (6)
12	0.5 (8)	0.11 (9)	0.17 (6)
13	0.5 (6)	0.25 (8)	0.33 (6)
14	0.33 (6)	0.29 (7)	0.33 (6)
15	0.67 (6)	0.14 (7)	0.17 (6)
16	0.5 (4)	0.17 (6)	0.17 (6)
17	0 (4)	0.17 (6)	0.17 (6)
18	0 (3)	0 (5)	0.25 (4)
19	0.33 (3)	0 (4)	0 (2)
20	0.33 (3)	0 (4)	0 (2)
21	0 (2)	0 (4)	0 (1)
22	0.5 (2)	0 (3)	
23	0 (2)	0 (3)	
24	0 (1)	0 (1)	
25	0 (1)	0 (1)	
26	0 (1)	0 (1)	
27		0 (1)	

The number in parentheses represents the number of thrips per cohort alive on that day.

The likelihood of transmission increasing with the amount of feeding per day differed according to parasitism status of the thrips. For non-parasitized females and males, the likelihood of transmission did not increase with their amount of feeding each day. The regression slopes were not significantly greater than 0 for non-parasitized females (t = 1.31, d.f. = 89, P = 0.10) or for non-parasitized males (t = 0.94, d.f. = 89, P = 0.18). In contrast, the likelihood of parasitized females transmitting on a particular day increased with their amount of feeding (t = 1.87, d.f. = 80, P = 0.03).

Discussion

The comprehensive nature of this research allowed for comparisons in feeding and transmission rates to be made for both male and female thrips and provided insight into how *T. fuscum* may be modulating the physiology of its obligate *F. fusca* host. Feeding rates of female *F. fusca* were reduced nearly 65% by *T. fuscum* parasitism, a value that was similar to results from previous studies on *T. nicklewoodi* parasitizing *F. occidentalis* (Arthurs & Heinz, 2003; Lim & Van Driesche, 2004). Arthurs & Heinz (2003) proposed that parasitized female thrips fed less because developing *T. nickelwoodi* juveniles distended the abdomen and triggered stretch receptors of *F. occidentalis*. However, feeding rates of parasitized females were reduced immediately upon adult emergence and juveniles were not observed in the host hemocoel until much later. Furthermore, parasitism by *T. fuscum* did not appear to affect the feeding rates of male thrips in our study. Although male *F. fusca* produced fewer *T. fuscum*, they are significantly smaller than females, and likely, fewer *T. fuscum* would distend their abdomen in an amount proportionate to parasitized females (Sims et al., 2005; Reitz et al., 2006). Because male thrips feeding behavior was not affected by *T. fuscum* parasitism, it is also unlikely that *T. fuscum* is disrupting host neurological systems or that stomach lesions resulting from the continual migration of *T. fuscum* progeny into and out of the alimentary tract reduce the host's ability to ingest food. A much more likely hypothesis is that *F. fusca* may be diverting nutrients from egg production towards sustaining *Thripinema* development (Hurd, 1990). Previous studies show that adult diet affects egg production of thrips and more energy (i.e., food intake) is likely needed for thrips oogenesis than is needed for sustaining *T. fuscum* infection (Teulon & Penman, 1991).

Despite the fact that *T. fuscum* reduced the amount of feeding by female *F. fusca*, parasitism did not decrease their longevity. Still *T. fuscum* parasitism had pathological effects on males, as parasitized males had lower survivorship than non-parasitized males despite feeding at similar levels. Lim et al. (2001) suggested that because healthy male thrips feed less than females, their limited nutrient reserves are depleted quicker when parasitized by *Thripinema* thus, reducing their longevity.

Tomato spotted wilt virus infection does not appear to have a significant effect on *F. fusca* longevity, which supports the findings of Wijkamp et al. (1996a) and Arthurs & Heinz (2003) for *F. occidentalis*. Our results suggest that viruliferous adult *F. fusca* have a delayed onset of mortality, and this phenomenon may be an adaptive strategy of TSWV to enhance transmission by its host. The efficiency with which thrips transmit TSWV decreased with age. Van de Wetering et al. (1999) suggested that TSWV transmission is a function of food ingestion; a higher consumption rate is associated with more viral particles being egested into plant tissue. Because feeding rates were consistent over the course of the thrips lifetime but transmission declined in our study, we hypothesize that viral titer may be reduced in older thrips because of senescence of salivary glands and/or degradation of viral particles.

In our study, non-parasitized females transmitted TSWV more efficiently than non-parasitized males. Sakurai et al. (1998) and van de Wetering et al. (1998, 1999) reported that male *F. occidentalis* transmit with a higher efficiency than females because of differences in feeding behavior. Females tend to feed more frequently and for longer intervals, which irreversibly destroys cell contents and can prevent viral replication within the target plant. In contrast, males feed with a higher frequency of shallow probing and induce only minor cell damage so the cells are better able to support viral infection. The difference in transmission efficiencies between genders in our study and those mentioned may be due to differences in thrips species and host plants used, as both Sakurai et al. (1998) and van de Wetering et al. (1998, 1999) tested the effects of TSWV transmission by *F. occidentalis* on *Datura stramonium* (L.).

Thripinema fuscum parasitism reduced TSWV transmission of adult female *F. fusca* by approximately 50%. Non-parasitized females fed more and had much higher transmission rates than parasitized females. This difference in transmission remained relatively constant throughout adulthood, which emphasizes the permanent impact of parasitism on thrips vector competence. Prior virus transmission data (Sakimura, 1963) has shown transmission of TSWV by viruliferous *F. fusca* adults to be sporadic. Transmissions for both non-parasitized and parasitized females in our study were sporadic and the gap times between transmissions were greater for parasitized females. We initially suspected vector capabilities would be reduced because *T. fuscum* lowers host feeding, and by doing this, reduces the viral titers delivered into plant tissue. This trend was observed when comparing the feeding and transmission rates for males and females: females fed more and transmitted at a higher rate than males. However, non-parasitized males fed less than parasitized females but transmitted TSWV at similar rates, suggesting other mechanisms may be operating to reduce transmission by parasitized females. Our results from the logistic analysis may indicate that parasitism not only reduces feeding (which indirectly affects transmission), but also that parasitism has some type of direct effect on virus replication. It may be that *T. fuscum* is sequestering important nutrients from the host that are required for successful development, and by doing so, alters the physiology of salivary gland cells and their ability to replicate virus. Alternatively, it has been shown that activation of the immune system of *F. occidentalis* by TSWV infection induces the up-regulation of antimicrobial and other immune system-related proteins (Medeiros et al., 2004). It is possible that both parasitism and TSWV infection may be inducing a synergistic immune response in the thrips that is detrimental to TSWV development.

Allantonematid nematodes induce various behavioral and physiological changes in their insect hosts (Hurd, 1993). By sterilizing female *F. fusca*, *T. fuscum* aids in reducing secondary spread of TSWV under field conditions (Sims et al., 2005). By reducing feeding, *T. fuscum* also aids in reducing primary spread of TSWV. Further studies are underway to determine how the parasitic *Thripinema* modulates the physiology of its thrips host. Understanding how these alterations influence vector competence may one day provide targets for suppressing disease spread.

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References

Ananthakrishnan TN & Annadurai RS (2007) Thrips–tospovirus interactions: biological and molecular implications. Current Science 92: 1083–1086.

Arthurs S & Heinz KM (2003) Thrips parasitic nematode *Thripinema nicklewoodi* (Tylenchida: Allantonematidae) reduces feeding, reproductive fitness, and tospovirus transmission by its host, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Environmental Entomology 32: 853–858.

Bandla MD, Westcot DM, Chenault KD, Ullman DE, German TL & Sherwood JL (1994) Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of Tomato spotted wilt tospovirus to identify viruliferous thrips. Phytopathology 84: 1427–1431.

Chamberlin JR, Todd JW, Beshear RJ, Culbreath AK & Demski JW (1992) Overwintering hosts and wingform of thrips, *Frankliniella* spp., in Georgia (Thysanoptera: Thripidae): implications for management of spotted wilt disease. Environmental Entomology 21: 121–128.

Culbreath AK, Todd JW & Brown SL (2003) Epidemiology and management of Tomato spotted wilt in peanut. Annual Review of Phytopathology 41: 53–75.

Funderburk J, Stavisky J, Tipping C, Gorbet D, Momol T & Berger R (2002) Infection of *Frankliniella fusca* (Thysanoptera: Thripidae) in peanut by the parasitic nematode *Thripinema fuscum* (Tylenchidae: Allantonematidae). Environmental Entomology 31: 558–563.

Groves RL, Walgenbach JF, Moyer JW & Kennedy GG (2003) Seasonal dispersal patterns of *Frankliniella fusca* (Thysanoptera: Thripidae) and Tomato spotted wilt virus occurrence in central and eastern North Carolina. Journal of Economic Entomology 96: 1–11.

Hunter WB & Ullman DE (1989) Analysis of mouthpart movements during feeding of *Frankliniella occidentalis* (Pergande) and *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae). International Journal of Insect Morphology and Embryology 18: 161–172.

Hurd H (1990) Parasite induced modulation of insect reproduction. Advances in Invertebrate Reproduction (ed. by M Hoshi & O Yamashita), pp. 163–168. Elsevier, Amsterdam, The Netherlands.

Hurd H (1993) Reproductive disturbances induced by parasites and pathogens. Parasites and Pathogens of Insects (ed. by NE Beckage, SN Thompson & BA Federici), pp. 87–105. Academic Press, San Diego, CA, USA.

Johnston G & So Y (2003) Analysis of data from recurrent events. Proceedings of the Twenty-Eighth Annual SAS® Users Group International Conference, Paper 273-28. SAS Institute, Cary, NC, USA.

Kerguelen V & Hoddle MS (1999) Measuring mite feeding damage on avocado leaves with automated image analysis software. Florida Entomologist 82: 119–122.

Lewis T (1997) Major crops infested by thrips with main symptoms and predominant injurious species (Appendix II). Thrips as Crop Pests (ed. by T Lewis), pp. 675–709. CAB International, New York, NY, USA.

Lim UT & Van Driesche RG (2004) Impatiens necrotic spot virus infection and feeding behavior of nematode-parasitized western flower thrips. Biological Control 31: 438–444.

Lim UT, Van Driesche RG & Heinz KM (2001) Biological attributes of the nematode, *Thripinema nicklewoodi*, a potential biological control agent of western flower thrips. Biological Control 22: 300–306.

Madden LV, Turechek WW & Nita M (2002) Evaluation of generalized linear mixed models for analyzing disease incidence data obtained in designed experiments. Plant Disease 86: 316–325.

Maris PC, Joosten NN, Goldbach RW & Peters D (2004) Tomato spotted wilt virus infection improves host suitability for its vector *Frankliniella occidentalis*. Phytopathology 94: 706–711.

Medeiros RB, Resende RO & deAvila AC (2004) The plant virus Tomato spotted wilt Tospovirus activates the immune system of its main insect vector, *Frankliniella occidentalis*. Journal of Virology 78: 4976–4982.

Nelson W (2003) Recurrent Events Data Analysis for Product Repairs, Disease Recurrences, and Other Applications. SIAM, Philadelphia, PA, USA.

Parrella G, Gognalons P, Gebre-Selassie K, Vovlas C & Marchoux G (2003) An update of the host range of Tomato spotted wilt virus. Journal of Plant Pathology 85: 227–264.

Reitz SR, Funderburk JE & Waring SM (2006) Differential predation by the generalist predator *Orius insidiosus* on congeneric species of thrips that vary in size and behavior. Entomologia Experimentalis et Applicata 119: 179–188.

Sakimura K (1963) *Frankliniella fusca*, an additional vector for the Tomato spotted wilt virus, with notes on *Thrips tabaci*, another vector. Phytopathology 53: 412–415.

Sakurai T, Murai T, Maedi T & Tsumuki H (1998) Sexual differences in transmission and accumulation of Tomato spotted wilt virus in its insect vector *Frankliniella occidentalis* (Thysanoptera: Thripidae). Applied Entomology and Zoology 33: 583–588.

SAS (2004) SAS/STAT Software, Version 9.1. SAS Institute, Cary, NC, USA.

SAS (2006) SAS/STAT: The GLIMMIX Procedure. SAS Institute, Cary, NC, USA.

Sharga US (1932) A new nematode, *Tylenchus aptini* n. sp. parasite of Thysanoptera (Insecta: *Aptinothrips rufus* Gmelin). Parasitology 24: 268–279.

Sims K, Funderburk J & Boucias D (2005) Host–parasite biology of *Thripinema fuscum* (Tylenchida: Allantonematidae) and *Frankliniella fusca* (Thysanoptera: Thripidae). Journal of Nematology 37: 4–11.

Stumpf CF & Kennedy GG (2005) Effects of Tomato spotted wilt virus isolates, host plants, and temperature on survival, size, and development time of *Frankliniella fusca*. Entomologia Experimentalis et Applicata 114: 215–225.

Stumpf CF & Kennedy GG (2007) Effects of Tomato spotted wilt virus isolates, host plants, and temperature on survival, size, and development time of *Frankliniella occidentalis*. Entomologia Experimentalis et Applicata 123: 139–147.

Teulon DAJ & Penman DR (1991) Effects of temperature and diet on oviposition rate and development time of the New Zealand flower thrips, *Thrips obscuratus*. Entomologia Experimentalis et Applicata 60: 143–155.

Tipping C, Nguyen KB, Funderburk JE & Smart GC (1998) *Thripinema fuscum* n. sp. (Tylenchida: Allantonematidae), a parasite of the tobacco thrips, *Frankliniella fusca* (Thysanoptera). Journal of Nematology 30: 232–236.

Ullman DE, German TL, Sherwood JL, Westcot DM & Cantone FA (1993) Tospovirus replication in insect vector cells: immuno-cytochemical evidence that the non structural protein encoded by the S RNA of Tomato spotted wilt Tospovirus is present in thrips vector cells. Phytopathology 83: 986–991.

van de Wetering F, Goldbach R & Peters D (1996) Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900–905.

van de Wetering F, Hulshof J, Posthuma K, Harrewijn P, Goldbach R & Peters D (1998) Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. Entomologia Experimentalis et Applicata 88: 9–15.

van de Wetering F, van der Hoek M, Goldbach R & Peters D (1999) Differences in Tomato spotted wilt virus vector competency between males and females of *Frankliniella occidentalis*. Entomologia Experimentalis et Applicata 93: 105–112.

Whitfield AE, Ullman DE & German TL (2005) Tospovirus-thrips interactions. Annual Review of Phytopathology 43: 459–489.

Wijkamp I, van Lent J, Kormelink R, Goldbach R & Peters D (1993) Multiplication of Tomato spotted wilt virus in its insect vector, *Frankliniella occidentalis*. Journal of General Virology 74: 341–349.

Wijkamp I, Goldbach R & Peters D (1996a) Propagation of Tomato spotted wilt virus in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. Entomologia Experimentalis et Applicata 81: 285–292.

Wijkamp I, van de Wetering F, Goldbach R & Peters D (1996b) Transmission of Tomato spotted wilt virus by *Frankliniella occidentalis:* median acquisition and inoculation access period. Annals of Applied Biology 129: 303–313.